Supplementary Material

CD4 T cells in *Mycobacterium tuberculosis* and *Schistosoma mansoni* co-infected individuals maintain functional TH1 responses

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1. Supplementary Figures

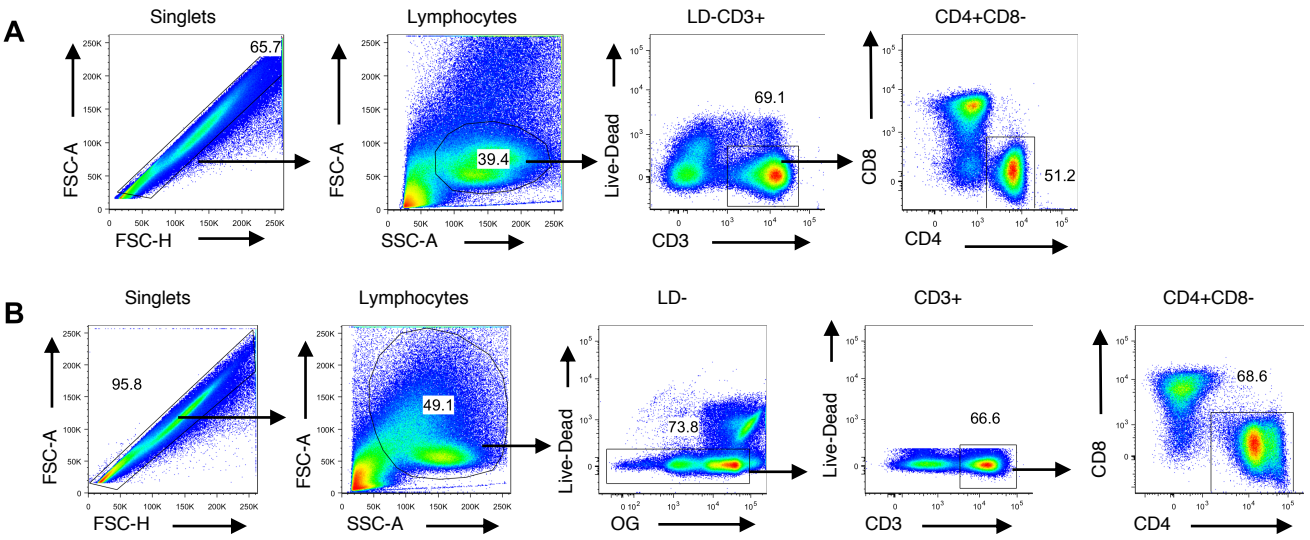

Figure S1. Gating strategy for flow cytometry analysis. (A) In this sample gating for the overnight ICS assay, cells were first gated for singlets (FSC-H vs. FSC- A) and lymphocytes (SSC-A vs. FSC-A). The lymphocyte gate is further analyzed for their uptake of the Zombie IR Live/Dead stain to determine live versus dead cells and their expression of CD3 (Zombie Near-IR^{lo}, CD3^{+}). CD4 and CD8 surface expression is then determined from this gated population. **(B)** In this sample gating for the Proliferation ICS assay, cells were first gated for singlets (FSC-H vs. FSC- A) and lymphocytes (SSC-A vs. FSC-A). The lymphocyte gate is further analyzed for their uptake of the Zombie IR Live/Dead stain to determine live versus dead cells (Zombie Near-IR^{lo}). Live cells are then gated for their expression of CD3 (CD3^{+}) and CD4 and CD8 surface expression is then determined from this gated population.

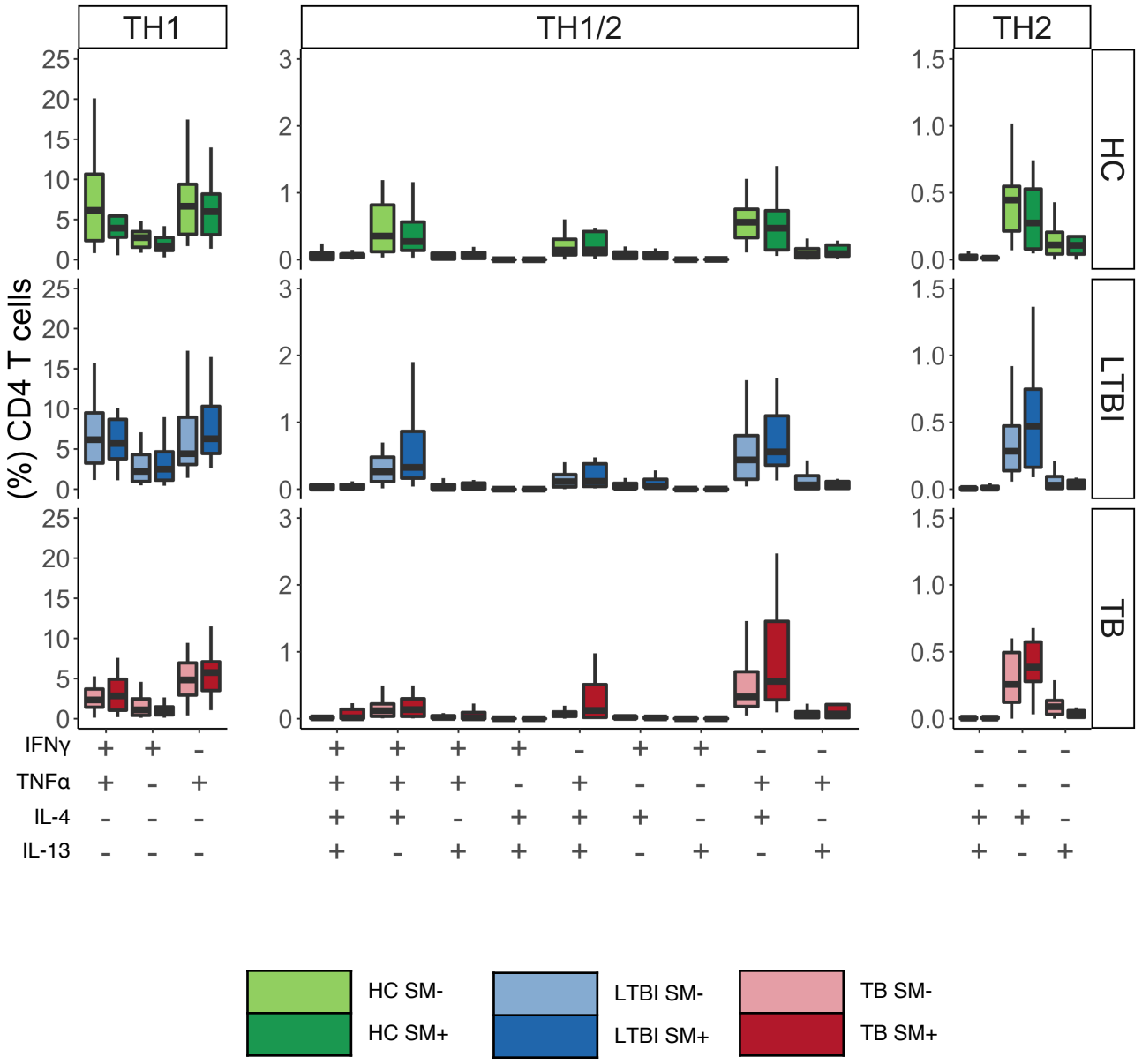

Figure S2. SM⁺ and SM⁻ individuals have similar frequencies of cytokine⁺ CD4 T cells across all combination of TH1 and TH2 cytokines. PBMC samples obtained from individuals in each of six groups defined by TB and *S. mansoni* infection status were incubated for 18 h in media alone (negative control) or stimulated with PMA and Ionomycin. Intracellular expression of IFNγ, TNFα, IL-4 and IL-13 was measured by flow cytometry (HC SM⁻, n=24; HC SM⁺, n=13; LTBI SM⁻, n=25; LTBI SM⁺, n=25; TB SM⁻, n=25; TB SM⁺, n=15). Frequency of each combination of cytokines using a Boolean gating strategy grouped by TH lineage. Data are shown after subtraction of background cytokine production in the unstimulated negative control condition. Boxes represent the median and interquartile ranges; whiskers represent 1.5*IQR. Differences in the frequency of each cytokine⁺ CD4 T cell population between SM⁺ and SM⁻ individuals were assessed using a Mann Whitney *U* test. P-values < 0.05 were considered significant.

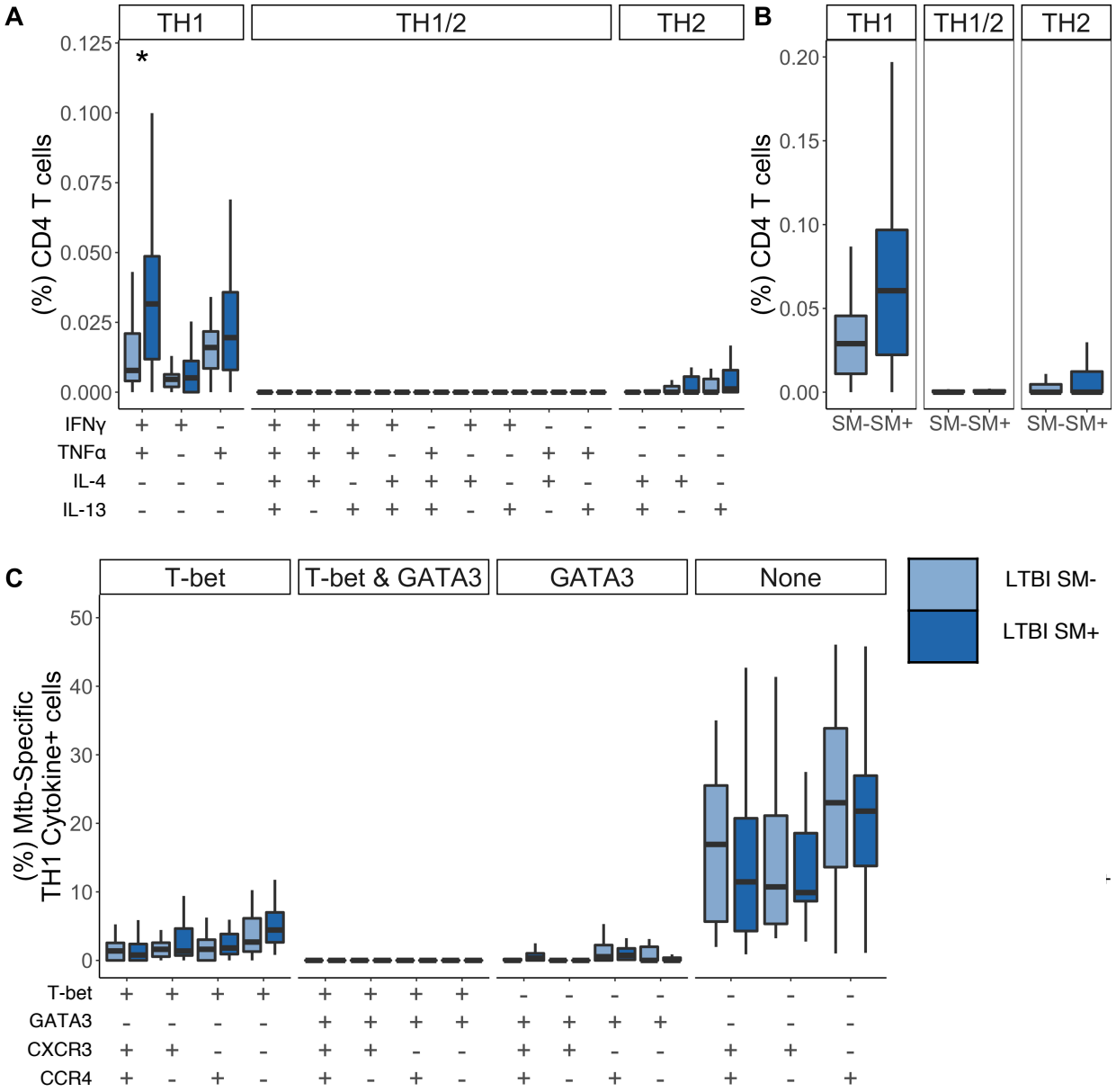

Figure S3. SM⁺ LTBI individuals have higher frequencies of IFNγ⁺TNFα⁺ Mtb-specific CD4 T cells, which express both TH1 and TH2 lineage markers. PBMC samples obtained from SM⁺ and SM⁻ LTBI individuals were stimulated for 18 h with Mtb peptides CFP-10 and ESAT-6. Intracellular expression of IFNγ, TNFα, IL-4 and IL-13 was measured by flow cytometry (SM⁻, n=24; SM⁺, n=22). **(A)** Frequency of each combination of cytokines using a Boolean gating strategy. (**B**) Frequency of each aggregated group of TH cytokine⁺ CD4 T cells as defined in S4A. Samples meeting the criteria for a positive response (see Materials and Methods) were evaluated for expression of lineage specific phenotypic markers using a Boolean gating strategy. (**C**) Frequency of each combination of transcription factors and chemokine receptors amongst TH1 cytokine⁺ CD4 T cells (SM⁻, n=16; SM⁺, n=18). Boxes represent the median and interquartile ranges; whiskers represent the 1.5*IQR. Differences in the frequencies of TH1, TH1/2, and TH2 CD4 T cells within each group were evaluated using a Kruskal Wallis test. TH1 cytokine frequencies were statistically higher than the both TH1/2 and TH2 frequencies after applying the Bonferroni correction for multiple comparisons. Differences in the frequency of each CD4 T cell population between SM⁺ and SM⁻ individuals were assessed using a Mann Whitney *U* test. **: p< 0.01; *:p< 0.05

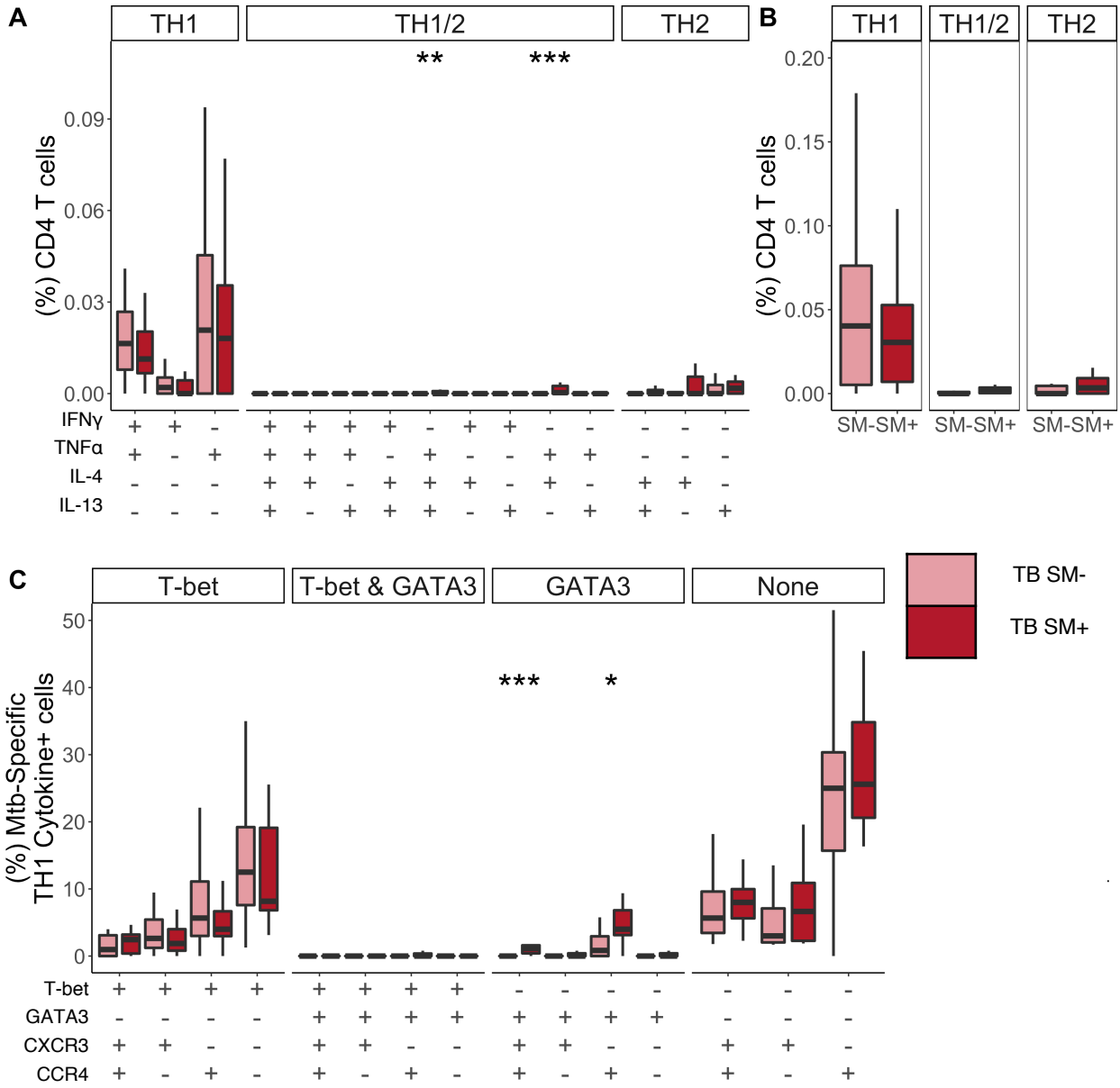

Figure S4. SM⁺ TB individuals have higher frequencies of IL-4⁺TNFα⁺ and GATA3⁺CCR4⁺ Mtb-specific CD4 T cells. PBMC samples obtained from SM⁺ and SM⁻ TB individuals were stimulated for 18 h with Mtb peptides CFP-10 and ESAT-6. Intracellular expression of IFNγ, TNFα, IL-4 and IL-13 was measured by flow cytometry (SM⁻, n=25; SM⁺, n=15). **(A)** Frequency of each combination of cytokines using a Boolean gating strategy. (**B**) Frequency of each aggregated group of TH cytokine⁺ CD4 T cells as defined in S5A. Samples meeting the criteria for a positive response (see Materials and Methods) were evaluated for expression of lineage specific phenotypic markers using a Boolean gating strategy. (**C**) Frequency of each combination of transcription factors and chemokine receptors amongst TH1 cytokine⁺ CD4 T cells (SM⁻, n=15; SM⁺, n=9). Boxes represent the median and interquartile ranges; whiskers represent the 1.5*IQR. Differences in the frequencies of TH1, TH1/2, and TH2 CD4 T cells within each group were evaluated using a Kruskal Wallis test. TH1 cytokine frequencies were statistically higher than the both TH1/2 and TH2 frequencies after applying the Bonferroni correction for multiple comparisons. Differences in the frequency of each CD4 T cell population between SM⁺ and SM⁻ individuals were assessed using a Mann Whitney *U* test. **: $p < 0.01$; *: $p < 0.05$

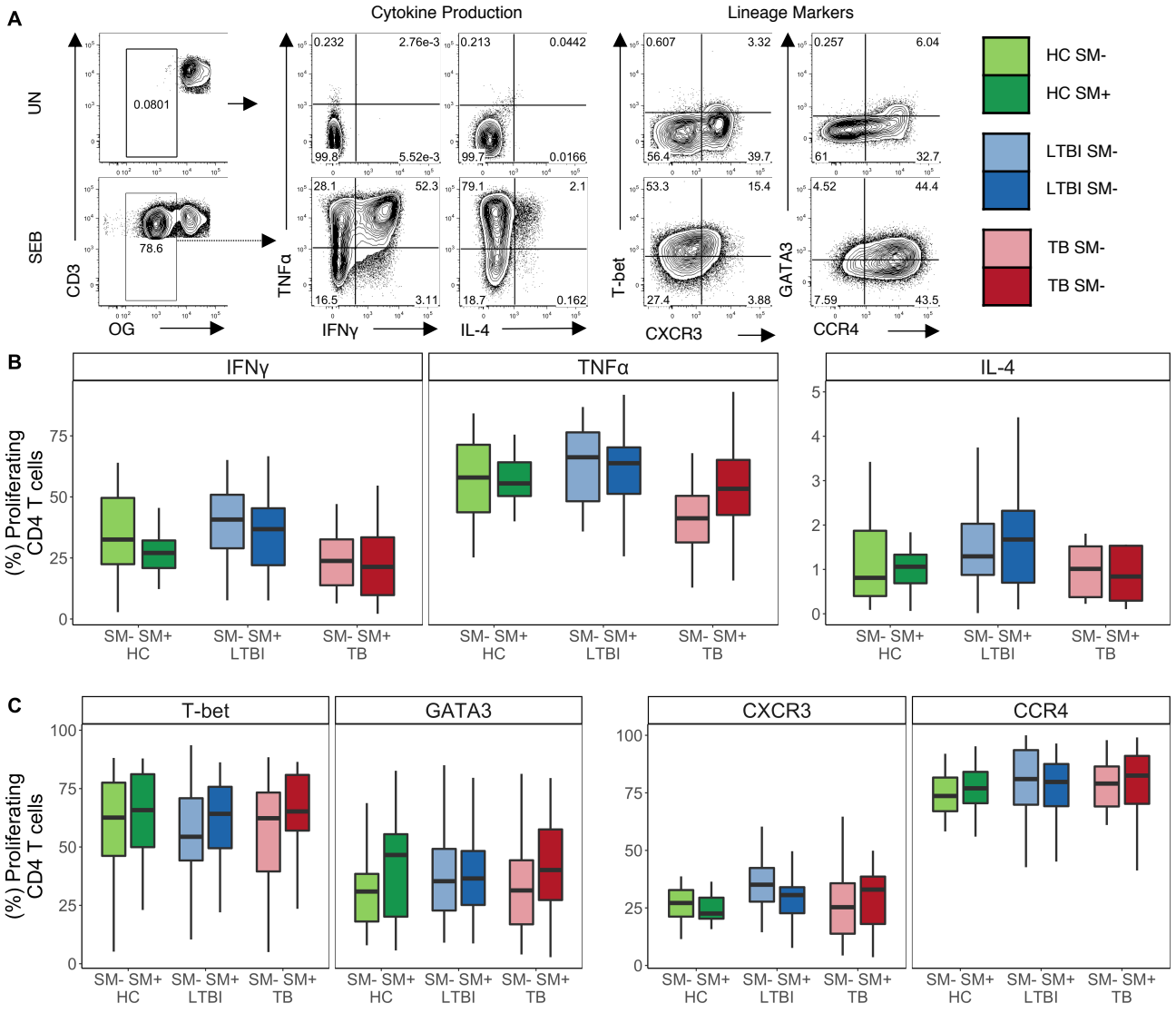

Figure S5. Proliferating CD4 T cells have equivalent expression of TH1 and TH2 cytokines and lineage markers in *S. mansoni*⁺ and *S. mansoni*⁻ individuals across Mtb infection groups. PBMC from SEB stimulated condition were restimulated on day 5 with PMA and Ionomycin for 5 hours to induce cytokine production. Samples meeting the criteria for a positive proliferative response (see Materials and Methods) were evaluated for cytokine production and expression of lineage specific transcription factors and chemokine receptors by flow cytometry. **(A)** Representative flow plots from an *S. mansoni*⁺ LTBI individual. Unstimulated samples (upper) show cytokine production and phenotypes on cells gated on live $CD3^{+}CD4^{+}CD8^{-}$ lymphocytes. SEB samples (lower) show cytokine production and phenotypes on cells gated on live $OG^{lo}CD3^{+}CD4^{+}CD8^{-}$ lymphocytes. **(B)** Frequency of TH1 cytokine⁺ and TH2 cytokine⁺ cells amongst proliferating CD4 T cells. **(C)** Frequency of transcription factor⁺ and chemokine⁺ cells amongst proliferating CD4 T cells. Boxes represent the median and interquartile ranges; whiskers represent the minimum and maximum 1.5*IQR. Differences in the frequency of each CD4 T cell population between *S. mansoni*⁺ and *S. mansoni*⁻ individuals were assessed using a Mann Whitney *U* test. *P-values* < 0.05 were considered significant.

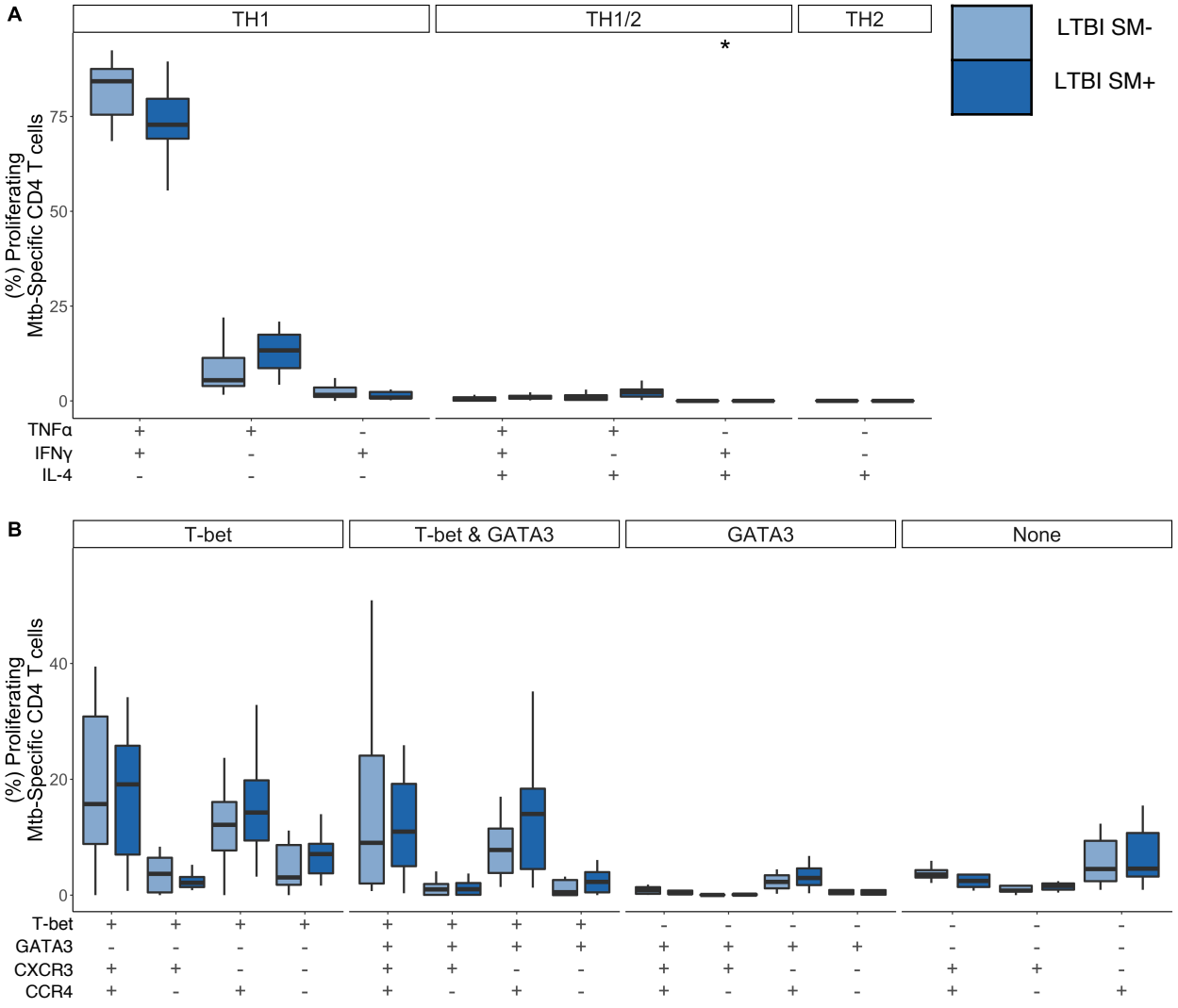

Figure S6. Proliferating Mtb-specific CD4 T cells produce TH1 cytokines and express both TH1 and TH2 lineage markers in SM$^+$ and SM$^-$ LTBI individuals. PBMC from the CFP-10 and ESAT-6 stimulated condition were restimulated on day 5 with PMA and Ionomycin for 5 hours to induce cytokine production. Samples meeting the criteria for a positive proliferative response (see Materials and Methods) were evaluated for cytokine production and expression of lineage specific transcription factors and chemokine receptors by flow cytometry (SM$^-$, n=10; SM$^+$, n=11). **(A)** Frequency of each combination of TH1 and TH2 cytokine$^+$ cells amongst proliferating CD4 T cells. **(B)** Frequency of each combination of transcription factor$^+$ and chemokine receptor$^+$ cells amongst proliferating CD4 T cells. Boxes represent the median and interquartile ranges; whiskers represent the 1.5*IQR. Differences in the frequency of each CD4 T cell population between SM$^+$ and SM$^-$ individuals were assessed using a Mann Whitney *U* test. *:$p < 0.05$